Green Synthesis of silver nanoparticles using *Aloe barbadensis Miller* Leaf Extract and it's antimicrobial activity

Project report submitted to Sister Nivedita University in partial fulfilment of the requirement for the degree of

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IN
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The dissertation work was carried out as a part of the academic requirement for the partial fulfillment of the degree of Master of Science in Biotechnology under the School of Health Science and Translational Research, Sister Nivedita University, Kolkata.

The research project entitled *"Green Synthesis of silver nanoparticles using Aloe barbadensis Miller Leaf Extract and it's antimicrobial activity"* was conducted during the period January 2025 to June 2025, and the student has shown sincere dedication and scientific aptitude throughout the course of the work.

I wish the student every success in future academic and professional endeavors.

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Abbreviations

- AgNPs – Silver Nanoparticles
- FTIR – Fourier Transform Infrared Spectroscopy
- DLS – Dynamic Light Scattering
- XRD – X-ray Diffraction
- UV-Vis – Ultraviolet-Visible Spectroscopy

ABSTRACT

The research and development of new, long-lasting substitutes to standard antibiotics is necessary in light of the growing issue of antimicrobial resistance (AMR). Silver nanoparticles, or AgNPs, are potent antibacterial agents owing to their broad-spectrum activity and unique physicochemical properties. In this work, the green synthesis of AgNPs is investigated using the aqueous extract of *Aloe barbadensis Miller*, usually referred to as aloe vera, a plant rich in bioactive phytochemicals like flavonoids, phenolic acids, terpenoids, and polysaccharides. By acting as organic reducing and capping agents, these compounds make the production of AgNPs more affordable and beneficial to the environment.

The synthesis process was visually confirmed by a distinct hue shift, and spectroscopic validity was provided by UV-Vis analysis, which showed a surface plasmon resonance peak at 425 nm. Additional characterization using X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), and dynamic light scattering (DLS) confirmed the amorphous or nanocrystalline nature of the generated nanoparticles in addition to their stable colloidal properties. The antibacterial activity of aloe vera-mediated AgNPs was evaluated against both Gram-positive (*Bacillus subtilis, Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria using agar well diffusion and broth microdilution assays. This study demonstrated a dose-dependent increase in bacterial inhibition along with a notable leakage of intracellular proteins and DNA, demonstrating membrane disruption as a critical antibacterial mechanism.

This work illustrates how aloe vera can be applied as a reducing agent and a biofunctional enhancer in the synthesis of nanoparticles, providing a biocompatible and environmentally friendly platform for antimicrobial applications. The research bolsters the possibility of incorporating plant-mediated AgNPs into agricultural biopesticides, wound dressings, water purification systems, and biomedical devices, thereby advancing sustainable nanotechnology solutions to counteract microbiological threats.

Keywords:

Green synthesis, Silver nanoparticles (AgNPs), *Aloe barbadensis Miller*, Antimicrobial activity, Phytochemicals, Biocompatibility, Nanotechnology.

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Introduction

Nanotechnology, or the manipulation of matter at the atomic and molecular level, has revolutionized several scientific fields of study, most notably environmental studies, materials engineering, and medicine. Among the various nanomaterials being studied, silver nanoparticles (AgNPs) have drawn a lot of interest due to their remarkable physicochemical properties. Strong antimicrobial activity against a variety of microorganisms is the most important of these, along with a high surface area-to-volume ratio, improved catalytic activity, and extraordinary chemical stability. Food packaging, textiles, water purification systems, wound dressings, and antimicrobial coatings for medical equipment are just a few of the products that presently use AgNPs. (Duman et al., 2024)

Although silver nanoparticles have been produced since ancient times, their systematic documentation did not occur until the late 19th and early 20th centuries. Ancient societies were aware of their use and employed silver nanoparticles in various applications. One notable example is the Lycurgus Cup, created by Roman glassmakers, which changes color due to the presence of embedded gold and silver nanoparticles. (Freestone et al., 2008)

Milestone	Contributor	Year	Significance
Colloidal metal nanoparticles (gold)	Michael Faraday	1857	Laid the groundwork for nanoparticle research
Chemical synthesis of silver nanoparticles	Creighton et al.	1979	Utilizing reducing agents, the first modern-day synthesis

Table1.1: **Milestones in nanoparticle research.**

Nanoparticles (NPs) are materials with at least one dimension in the nanometer range, typically between 1 and 100 nanometers (nm). At this scale, materials exhibit unique physicochemical properties due to their high surface area-to-volume ratio, increased surface energy, and quantum confinement effects. These characteristics lead to distinct optical, electrical, magnetic, catalytic, and biological properties when compared to their bulk counterparts. Metals (like silver, gold, and zinc), metal oxides (such as TiO_2 and ZnO), polymers, lipids, and ceramics are some of the materials that can make up nanoparticles. Among these, metallic nanoparticles—in particular, silver nanoparticles, or AgNPs—have drawn a lot of interest because of their strong antibacterial,

antiviral, anti-inflammatory, and wound-healing capabilities. Because of these qualities, they are very valuable in industries like agriculture, food technology, environmental science, biomedicine, and cosmetics. (Abbas et al., 2024)

Importance of AgNPs in Antimicrobial Applications: Antimicrobial resistance (AMR) is a substantial and extending global health issue where harmful microorganisms, such as bacteria, fungi, and viruses, develop the ability to resist exposure to drugs that are used to effectively eradicate them. This resistance results from genetic changes and selection pressure caused by the overuse or misuse of antibiotics in agriculture, medicine, and animal husbandry. Treatment failures, recurrent infections, increased hospital stays, and higher death rates are just a few of the serious outcomes of AMR. (Ferraz, 2024)

AMR is one of the top ten worldwide threats to public health, according to the World Health Organization. Traditional antibiotics are becoming less effective, so researchers are investigating new antimicrobial medications. Interest has been sparked by the potent antibacterial qualities of silver nanoparticles (AgNPs), especially those made with plant extracts and sustainable production methods. In contrast to conventional antibiotics, AgNPs target multiple biological product pathways, such as disrupting DNA replication, causing reactive oxygen species (ROS), and rupturing cell membranes, all of which make it harder for bacteria to develop resistance. This multifunctional mechanism offers an alternative option in the fight against drug-resistant infections. (Tahmasebi et al., 2025)

Techniques for Nanoparticle Synthesis:

The two main approaches used for creating nanoparticles are top-down and bottom-up. To reduce bulk materials to nanoscale particles, top-down processes are applied. While processes like ball milling use mechanical force to grind materials, laser ablation uses a concentrated laser in a liquid solution to evaporate metal surfaces. Regardless of their simplicity, those techniques often do not provide precise control over the particle's size and shape. (Roy et al., 2024)

The bottom-up method uses atomic or molecular precursors to create nanoparticles. Techniques such as co-precipitation, microemulsion, and chemical reduction provide better control over the uniformity of the particles. However, they may involve potentially hazardous chemical processes, like the sol-gel method (Abbas et al., 2024b)

In contrast, green synthesis techniques are gaining popularity due to their environmental benefits. These biological processes utilize bacteria, fungi, or plant extracts to naturally reduce metal ions.

For example, phytochemicals in aloe vera extract serve as both reducing and stabilizing agents during the synthesis of silver nanoparticles. This method is sustainable, biocompatible, and effective against bacteria. Each synthesis technique has its advantages, depending on the intended application and the specific properties desired in the particles. (Alwhibi et al., 2021a)

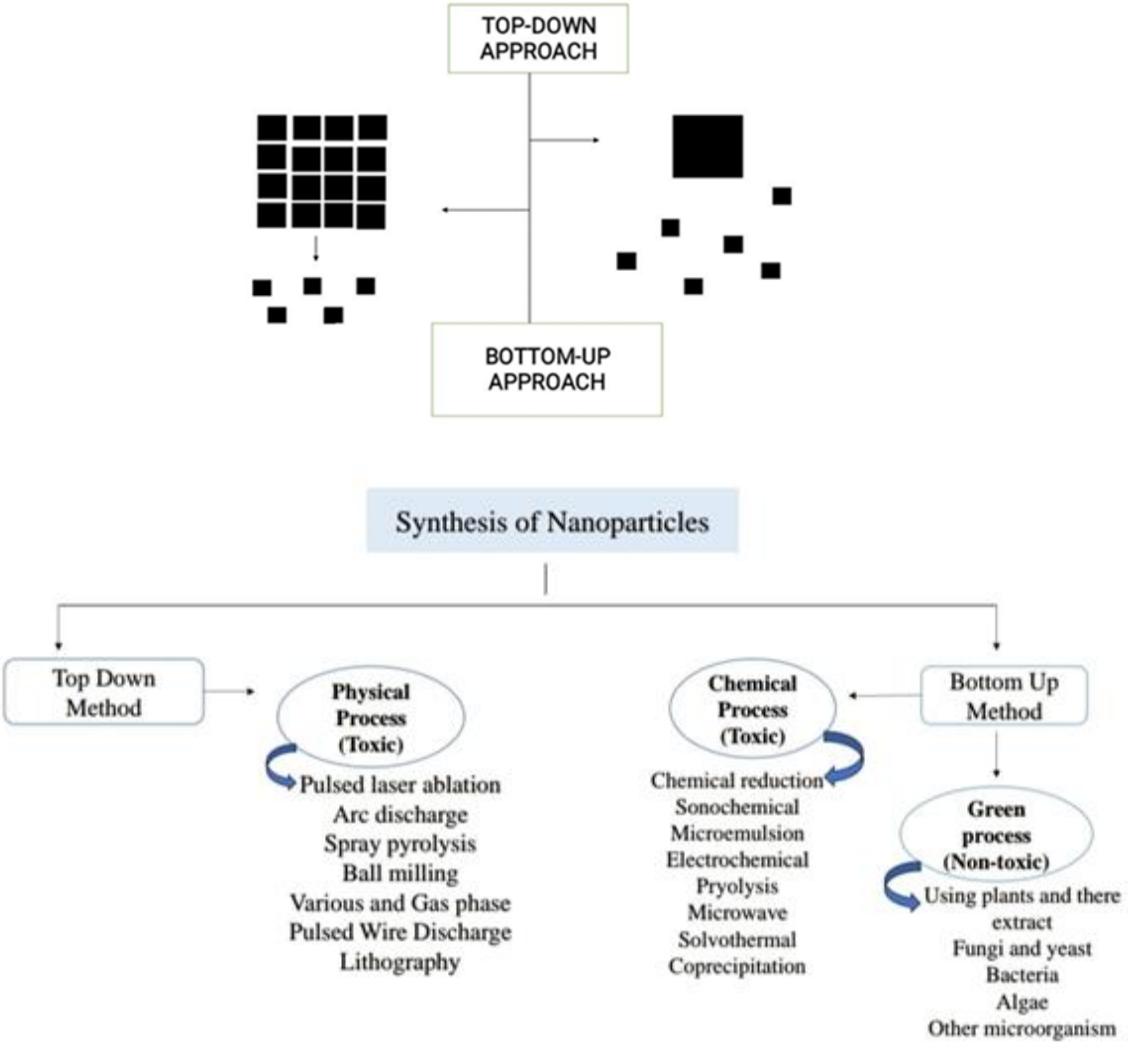


Fig 1. Categorization of nanoparticle synthesis techniques. Nanoparticles can be formed in two primary ways: top-down and bottom-up. The Top-Down method breaks down bulk materials into nanoscale structures through physical or chemical processes, which are typically dangerous. In contrast, the Bottom-Up approach generates nanoparticles from atomic or molecular precursors and can employ hazardous chemical processes or environmentally friendly green synthesis routes. By employing fungi, bacteria, algae, plant

extracts (like Aloe vera), and other microorganisms, the green method offers a non-toxic and sustainable alternative to the production of nanoparticles.

Nanoparticles organized them into different categories:

Type of Nanoparticle	Composition	Essential Characteristics	Applications	Nanotechnology Relevance
Metal Nanoparticles	Silver (Ag), Gold (Au), Copper (Cu)	Antimicrobial, Optical (SPR), Conductive	Antibacterial agents, Imaging, Sensors	Nano-optics, Biomedical nano-sensors
Metal Oxide Nanoparticles	ZnO, TiO_2, Fe_3O_4	Photocatalytic, Magnetic, UV-absorbing	Sunscreens, MRI contrast, Wastewater treatment	Environmental nanotech, Magnetic nanotech
Carbon-Based Nanoparticles	CNTs, Fullerenes, Graphene	High strength, Conductivity, Lightweight	Electronics, Drug delivery, Composite materials	Nanoelectronics, Structural nanomaterials
Polymeric Nanoparticles	PLGA, PEG-based polymers	Biodegradable, Biocompatible, Controlled release	Drug delivery, Vaccine carriers	Pharmaceutical nanotech
Lipid-Based Nanoparticles	SLNs, Liposomes, NLCs	Biocompatibility, Targeted release	Nutraceuticals, Cosmetics, Drug delivery	Nano-pharmaceutical formulations
Quantum Dots	CdSe, PbS (semiconductors)	Size-tunable fluorescence, Optical stability	Bioimaging, Solar cells, Diagnostics	Nano-optics, Nanoimaging
Dendrimers	Synthetic branched polymers	High functionality, Nanoscale size	Targeted drug/gene delivery, Imaging	Biomedical nanotechnology
Ceramic Nanoparticles	Silica, Alumina	High stability, Porosity	Bone regeneration, Catalysis	Biomedical and catalytic nanotech
Magnetic Nanoparticles	Fe_3O_4, $CoFe_2O_4$	Magnetic responsiveness, Biocompatibility	MRI, Targeted therapy, Data storage	Magnetic nanotechnology, Medical nanodevices
High-Entropy Alloy Nanoparticle	Multi-metallic systems	Thermal stability, Catalytic synergy	Catalysis, Energy storage	Advanced materials nanotechnology

Table 1.2: **Classification of various nanoparticles based on their material composition, characteristics, and major applications in nanotechnology**.

Conventional synthesis methods for AgNPs, including chemical reduction, electrochemical techniques, and physical approaches like laser ablation, are effective in producing nanoparticles with controlled size and shape.(Kedar et al., 2022) However, these methods often require hazardous chemicals, high energy input, and sophisticated laboratory infrastructure. Such limitations not only increase production costs but also raise environmental and health concerns due to toxic by-products and non-biodegradable residues. (Vishwanath & Negi, 2021)

Green synthesis has gained popularity as an effective and long-term solution for these issues. This method uses the reducing and stabilising qualities of biological entities, such as bacteria, fungi, algae, and plant extracts, to produce nanoparticles in mild, eco-friendly environments.(Ansari et al., 2023) Among these biological sources, plant-mediated synthesis is unique due to its simplicity, cost-effectiveness, scalability, and a wide range of phytochemicals found in plants that act as organic reducing and capping agents. (Shahzadi et al., 2025)

Importance of AgNPs in Antimicrobial Applications: Antimicrobial resistance (AMR) is a substantial and extending global health issue where harmful microorganisms, such as bacteria, fungi, and viruses, develop the ability to resist exposure to drugs that are used to effectively eradicate them. This resistance results from genetic changes and selection pressure caused by the overuse or misuse of antibiotics in agriculture, medicine, and animal husbandry. Treatment failures, recurrent infections, increased hospital stays, and higher death rates are just a few of the serious outcomes of AMR. (Krishnaprasad & Kumar, 2024; Petersen et al., 2023)

Aloe vera (*Aloe barbadensis Miller*), a succulent species of the Liliaceae family that has been used for centuries in traditional medicine across many cultures, is one plant that has demonstrated extraordinary promise in this area.(Arshad et al., 2022a) Aloe vera is well known for a broad range of therapeutic properties, notably its immunomodulatory, anti-inflammatory, antimicrobial, antioxidant, and wound-healing effects. Its rich phytochemical profile—which includes anthraquinones (like aloin and emodin), flavonoids, saponins, tannins, phenolic compounds, enzymes, lignins, and polysaccharides like acemannan—is principally accountable for these biological activities. (Maan et al., 2025)

Aloe vera is an even more desirable bio-factory for the synthesis of nanoparticles due to its abundance, affordability, and renewable nature. Aloe vera-derived nanoparticles are best suited

for use in biomedical applications that include antibacterial formulations, materials for wound healing, drug delivery systems, and biosensors since the particles have been shown to preserve or enhance the plant's innate antimicrobial and antioxidant properties.(Koratala et al., 2023)

Fig 1.1. Aloe vera plant

Study Purpose and Importance: This study explores the environmentally friendly production of silver nanoparticles (AgNPs) and evaluates their antibacterial properties using leaf extract from *Aloe barbadensis Miller*.(Sharma et al., 2021) As part of the scope, aloe vera-mediated AgNPs are made, described, and biologically assessed against particular bacterial strains. The importance of this approach lies in offering a sustainable, cost-effective, and biocompatible alternative to conventional chemical synthesis methods. The study promotes green nanotechnology, which may find utilize in biomedicine, antimicrobial drug development, and environmental sanitation.
(Giri et al., 2022)

Review of Literature

The Natural Green Alchemists: The Synthesis of Nanoparticles by Plants:

Plant Name	Scientific Name	Types of Nanoparticles Synthesized	Key Bioactive Compounds
Neem	*Azadirachta indica*	Silver (Ag)	Flavonoids, terpenoids, tannins, azadirachtin
Tulsi (Holy Basil)	*Ocimum sanctum*	Silver (Ag), Gold (Au)	Eugenol, flavonoids, tannins, alkaloids
Green Tea	*Camellia sinensis*	Silver (Ag), Gold (Au)	Polyphenols (catechins), flavonoids
Aloe vera	*Aloe barbadensis miller*	Silver (Ag)	Anthraquinones, flavonoids, carbohydrates, saponins
Moringa	*Moringa oleifera*	Silver (Ag)	Ascorbic acid, flavonoids, phenolics
Eucalyptus	*Eucalyptus spp.*	Silver (Ag)	Tannins, flavonoids, terpenoids
Turmeric	*Curcuma longa*	Gold (Au)	Curcumin, flavonoids, phenolics
Ginger	*Zingiber officinale*	Silver (Ag)	Gingerol, shogaol, flavonoids

Table 2.1: **Medicinal plants used in green nanoparticle synthesis and their key bioactive compounds.** (Shuaibu et al., n.d.)

Numerous plants, including neem, tulsi, green tea, aloe vera, and turmeric, can naturally produce nanoparticles. Their extracts consist of compounds that assist in converting metal salts into nanoparticles of zinc, gold, and silver. We termed this a "green" or "plant-based" synthesis. (Vanlalveni et al., 2021)

Variety of Plants Used in AgNP Synthesis: An extensive study by (Islam, 2021) identified over 100 plant species from various families that are used in the biosynthesis of silver nanoparticles (AgNPs). These plants belong to several taxonomic groups, including Asteraceae, Fabaceae, Lamiaceae, and Rutaceae. Each part of the plant—such as leaves, stems, roots, flowers, seeds, peels, and even latex—provides unique phytochemicals that facilitate the formation of nanoparticles. (Alwhibi et al., 2021b)

For example, the study highlighted the effective production of AgNPs using extracts from:

- *Aloe vera* (Asphodelaceae)

- *Azadirachta indica* (Meliaceae)
- *Camellia sinensis* (Theaceae)
- *Catharanthus roseus* (Apocynaceae)
- *Curcuma longa* (Zingiberaceae)
- *Eclipta prostrata* (Asteraceae)
- *Ficus carica* (Moraceae)
- *Moringa oleifera* (Moringaceae)
- *Ocimum sanctum* (Lamiaceae)
- *Punica granatum* (Lythraceae)

These examples prove the vast potential of plant biodiversity in nanoparticle synthesis. (Fahim et al., 2024a; Vanlalveni et al., 2021b)

Green synthesis of AgNPs using Aloe vera Extract: The green synthesis of silver nanoparticles (AgNPs) using aloe vera extract is an interesting method that utilises the medicinal qualities of aloe vera to make AgNPs with antibacterial properties. Here's a more thorough explanation:

Aloe vera extract's plant-based elements. Aloe vera extract contains an extensive variety of phytochemicals, including:

Flavonoids are plant-derived compounds known for their reducing and antioxidant properties, which aid in converting silver ions into AgNPs.

Silver nanoparticles (AgNPs) remain stable and silver ions are diminished with the assistance of **phenolic** acids.

Terpenoids contribute to maintaining the precise size and shape of AgNPs by stabilizing and reducing them. (Kumar et al., 2025; Tippayawat et al., 2016a)

The Phytochemical Composition of Aloe Vera: Aloe vera's performance in producing nanoparticles is due to its high phytochemical content. Nearly 75 potentially active ingredients can be found in aloe vera gel, including: These polyphenols include anthraquinones, flavonoids, and tannins.

- Vitamins A, C, E, and B12
- The enzymes amylase, catalase, and lipase
- Saponins
- Sugars, or monosaccharides and polysaccharides
- The amino acids
- Minerals (calcium, magnesium, and zinc)

Silver nanoparticle stabilization, capping, and reduction are dependent on these bioactive substances. Such as phenolic compounds and flavonoids lead electrons to the decreased ratio of Ag ions to metallic Ag ions. As stabilizing agents, polysaccharides and saponins envelop the nanoparticles in a protective shell which increases dispersion and inhibits aggregation. (Alwhibi et al., 2021c)

It has long been acknowledged that aloe vera is a multipurpose plant with a variety of health advantages. Its tapered, prickly leaves grow from short branches close to the ground. To maximize effectiveness, it is generally recommended to use fresh aloe vera gel, as the active compounds can diminish over time. When applied to the skin, aloe vera gel creates an adhesive-like layer that acts as a natural bandage, allowing nutrients to penetrate while preventing bacteria and other harmful substances from interfering with the healing process. Moreover, aloe vera gel has a high water content, which is essential for the body's healing mechanisms. (Kumar et al., 2025; Tippayawat et al., 2016a)

Mechanism of Synthesis: When synthesizing silver nanoparticles (AgNPs) using aloe vera extract, the following steps are involved:

1. **Reduction of Silver Ions**: Silver ions (Ag+) are converted into silver nanoparticles (AgNPs) through the action of phytochemicals found in aloe vera extract. (Tippayawat et al., 2016b)

2. **Stabilization of AgNPs**: The same phytochemicals help stabilize the AgNPs by preventing them from aggregating and ensuring they maintain a uniform size and shape. (Arshad et al., 2022b)

Aloe Vera's Advantages for Green Synthesis:

a. **Eco-Friendly and Sustainable Technique** - The green synthesis using aloe vera reduces its environmental impact by eliminating the need for hazardous chemicals and energy-

intensive processes. It advances sustainable nanotechnology, which advances the Sustainable Development Goals (SDGs).

b. **Sustainability of the economy** - Aloe vera is easy to grow, readily available, and requires little processing. This makes the synthesis process scalable and inexpensive for large-scale applications. (Gangwar et al., 2025)
c. **Biocompatibility -** Silver nanoparticles (AgNPs) derived from aloe vera exhibit higher biocompatibility because they do not contain harmful chemicals. This is particularly important in biomedical applications such as antimicrobial coatings, wound healing, and drug delivery.
d. **Increased Stability** - Nanoparticles made from aloe vera demonstrate excellent colloidal stability over time. The presence of proteins and polysaccharides helps to prevent agglomeration and degradation, ensuring a long shelf life and consistent reproducibility.
e. **Physical Characteristics That Vary** - Researchers can effectively control the size, shape, and surface features of silver nanoparticles (AgNPs) by adjusting factors such as extract concentration, pH, and temperature. The rich abundance of bioactive compounds found in aloe vera enhances the ability to manipulate the characteristics of these nanoparticles.
f. **Antioxidant Properties** - The natural antioxidants present in aloe vera help reduce oxidative stress, which stabilizes AgNPs and enhances their biological activity. This property may be advantageous in therapeutic applications. (Qubtia et al., 2024; Taqveem Abdul Wali Khan et al., 2024a)

Role of Aloe vera in the synthesis of nanoparticles: Among the many plant sources, aloe vera (*Aloe barbadensis Miller*) has emerged as a viable option for green synthesis. The inner gel of aloe vera leaves is rich in bioactive substances, such as polysaccharides, anthraquinones, flavonoids, saponins, vitamins A, C, and E, and enzymes. These elements may operate as capping agents, stabilizing the nanoparticles, and reducing agents, transform Ag^{+} into Ag^{0}. (Qubtia et al., 2024)

When used to synthesize nanoparticles, aloe vera has numerous advantages. (Taqveem Abdul Wali Khan et al., 2024b)

- Non-toxic and biodegradable synthesis method

- dual function as a reducer and stabilizer
- numerous natural sources with firmly established medicinal benefits
- It is compatible with biological applications due to its inherent bioactivity. Mittal et al. (2013) illustrated the effectiveness and ease of use of the green synthesis process by using Aloe vera extract to yield spherical silver nanoparticles in a matter of minutes.

Aloe Vera and the Formation of Silver Nanoparticles (AgNPs): The physicochemical characteristics of silver nanoparticles (AgNPs), such as their size, shape, surface charge, and dispersion, significantly influence their biological activity. Aloe vera is known to produce AgNPs that typically possess the following features:

- Their shape is spherical or almost spherical.

- Their sizes range from 10 to 80 nanometers. (Logaranjan et al., 2016a)

- They demonstrate distributions that can be either monodispersed or slightly agglomerated, depending on the synthesis conditions. (Vélez et al., 2018a)

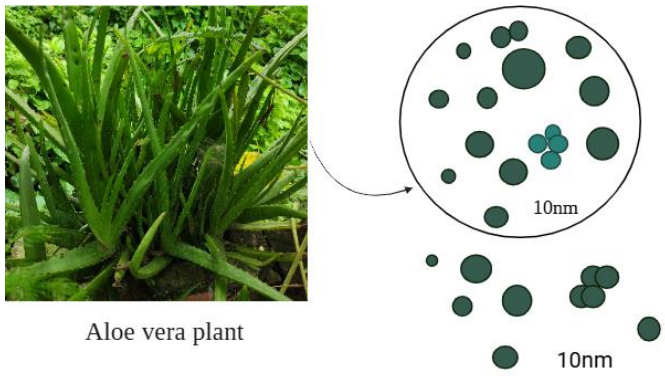


Fig 2. Aloe vera plant and schematic of synthesized nanoparticles (~10 nm) from its extract

Various characterization methods, including Fourier-transform infrared spectroscopy (FTIR), transmission electron microscopy (TEM), X-ray diffraction (XRD), and UV-visible spectroscopy, have been employed to confirm the formation of these nanoparticles and to identify the functional groups involved in their reduction and stabilization.

Additionally, AgNPs exhibit surface plasmon resonance (SPR), which is indicated by UV-Vis absorption peaks typically found between 400 and 450 nanometers.

Applications of Aloe Vera-Mediated Silver Nanoparticles: Silver nanoparticles produced using Aloe vera extract have a diverse range of applications due to their unique combination of physicochemical stability, biocompatibility, and potent biological activity, particularly their antibacterial properties. These characteristics enable Aloe vera-mediated silver nanoparticles (AgNPs) to be utilized in various fields, including environmental protection, medicine, and agriculture. (Riaz et al., 2022; Vélez et al., 2018a)

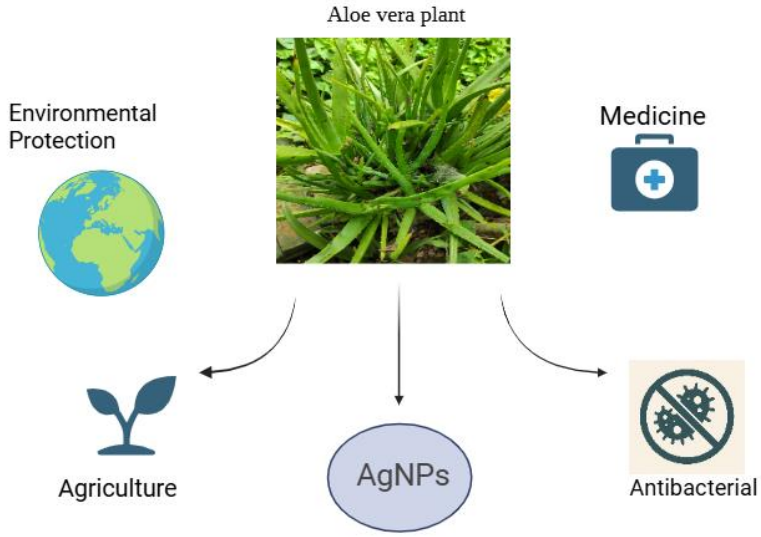


Fig 2.1. Illustrate the potential applications of silver nanoparticles (AgNPs) as antibacterial agents in agriculture, medicine, and environmental protection, utilizing an extract from the Aloe vera plant.

1. **Biomedical Applications**: Aloe vera-based AgNPs have special potential in the pharmaceutical and medical sectors due to their modified antibacterial, anti-inflammatory, antioxidant, and wound-healing properties. The natural source of aloe vera provides the nanoparticles' biocompatibility and reduced harm to cells compared to their chemically produced equivalents.

   (a) Antiseptic Protection and the Healing of Wounds -

   Using AgNPs in topical preparations to prevent infections in burns, ulcers, and wounds has gained detection. These nanoparticles have more effective healing effects because of aloe vera's anti-inflammatory and regenerative properties. Studies have shown that aloe vera-mediated AgNPs:

- Promote the growth of fibroblasts and collagen synthesis.
- Enhance re-epithelialization and angiogenesis.
- For prevention of wound infections, lessen microbial colonization.

Their features make them a valuable addition to ointments, sprays, and wound dressings. (Riaz et al., 2022; Vélez et al., 2018a)

Antiseptic protection and healing of wounds
Ag
Facilitate developement of fibroblasts and collagen
Enhance re - epithelization and angiogenesis
Reduce microbial colonization

Figure 2.2. Wound healing and antimicrobial effects of silver and plant-based topical formulation.

(b) Treatments with antibacterial and antifungal agents-

Because of their powerful bactericidal and fungicidal properties, aloe vera-AgNPs may be able to treat infections brought on by antibiotic-resistant bacteria, which include Candida albicans and Staphylococcus aureus (MRSA). Their ability to degrade biofilms, a major contributor to chronic infections, enhances their therapeutic potential.

(c) Aloe vera nanoparticles are excellent drug molecule carriers simply because of their small size, large surface area, and functional groups on the surface. Aloe vera-mediated AgNPs can be functionalized by using:

- Anticancer drugs (doxorubicin, for illustration)
- Antibiotics, such as ciprofloxacin
- Agents that minimize inflammation

These treatments' targeted and regulated release enhances the therapeutic effect while minimizing possible side effects. (Jiao et al., 2019; Rajak et al., n.d.)

(d) Cancer Treatment –

Aloe vera-synthesized silver nanoparticles (AgNPs) induce oxidative stress and apoptosis, leading to the death of various cancer cell lines, including breast cancer MCF-7 and colon cancer cells, based on early in vitro studies. These nanoparticles demonstrate selective toxicity to cancer cells while largely sparing healthy cells, making them a promising candidate for future anticancer therapies. (Vélez et al., 2018b)

1. **Applications in the Environment:** Aloe vera-mediated silver nanoparticles (AgNPs) offer promising options for environmental remediation, particularly in water treatment and surface disinfection. (Haripriya and Ajitha: Antimicrobial Efficacy of Silver Nanoparticles of Aloe Vera, n.d.)

(a) **Water Purification and Antimicrobial Filters** -

Thanks to their effective antibacterial properties, aloe vera-mediated AgNPs can be utilized in water filtration systems to eliminate harmful bacteria, viruses, and fungi. Silver nanoparticles embedded in membranes or immobilized on filter materials can significantly enhance the microbiological purity of drinking water.(Rodrigues et al., 2024)

These nanoparticles offer several advantages, including:

- Long-lasting antibacterial effectiveness

- Release of minimally harmful byproducts

- Prevention of biofouling

(b) **Surface Coatings for Antimicrobials –**

Aloe vera-derived AgNPs can be put on hospital surfaces, medical devices (such as implants and catheters), and even public touchpoints (which are referred as elevator buttons and doorknobs) to reduce the spread of microorganisms. The use of green-synthesised nanoparticles ensures minimized environmental toxicity in comparison to chemically assembled nanoparticles. (Spoiala et al., 2018)

2. **Utilization in Agriculture** - Aloe vera-derived AgNPs' antibacterial, antifungal, and growth-promoting properties make them more environmentally friendly than synthetic agrochemicals. (Fierascu et al., 2019)

    a) Management of Plant Environments -

    AgNPs can efficiently manage a range of phytopathogenic microorganisms that cause plant diseases:

    - Bacterial wilt (*Ralstonia solanacearum*)
    - Leaf spot in Alternaria spp.
    - Fusarium oxysporum, referred to as root rot

AgNPs derived from aloe vera perform as biopesticides by breaking down microbial membranes and preventing DNA replication, which reduces the need for chemical fungicides and bactericides.

b) Seed Germination and Plant Growth –

Aloe vera silver nanoparticles (AgNPs) can improve seed germination, promote root elongation, and increase biomass production in plants such as maize, wheat, and tomato when applied at low concentrations. These nanoparticles may interact with plant hormone mechanisms, leading to enhanced nutrient uptake.

However, it is important to note that AgNPs have a dose-dependent effect; high concentrations can be phytotoxic and detrimental to plant growth.

c) Packaging and Food Preservation –

The antibacterial qualities of aloe vera--mediated AgNPs make them suitable as active food packaging solutions that decrease microbiological spoiling and increase shelf life. These nanoparticles can be stated to:

- layers of chitosan and starch-based biodegradable polymer films.
- Edible fruit and vegetable wrappings
- Supplies for containers and wrapping

By preventing the growth of spoilage organisms like Salmonella enterica and Listeria monocytogenes, they help to ensure food safety and minimize waste. (Chaitanya Kumari et al., 2021)

Mechanisms of Antibacterial Activity of Silver Nanoparticles Produced by Aloe Vera: Extensive research has been conducted on the antibacterial properties of silver nanoparticles (AgNPs) mediated by Aloe vera. These nanoparticles have been shown to effectively treat several diseases caused by various microorganisms, including: (Tippayawat et al., 2016c)

- Gram-positive bacteria include Staphylococcus aureus and Bacillus subtilis.
- Gram-negative bacteria, contain Pseudomonas aeruginosa and Escherichia coli.
- Fungi, specifically Candida albicans.
- A significant number of drug-resistant strains.

Several complementary mechanisms are believed to contribute to the antimicrobial action of silver nanoparticles (AgNPs):

1. **Disruption of Cell Membranes:** AgNPs adhere to bacterial cell walls, which increases membrane permeability and allows the contents of the cells to leak out.

2. **Generation of Reactive Oxygen Species (ROS):** Nanoparticles induce oxidative stress, damaging lipids, proteins, and DNA within the cells.

3. **Deactivation of Proteins and Enzymes:** The interaction of AgNPs with thiol groups leads to the denaturation of essential enzymes.

4. **Interference with DNA Replication:** Silver ions released from nanoparticles inhibit cell division and disrupt the function of nucleic acids.

Research comparing pure silver nanoparticles to those synthesized with aloe vera suggests that compounds from aloe vera may enhance the antimicrobial effects of AgNPs.

The Impact of Synthesis Parameters: Several synthesis factors significantly affect the yield, stability, and biological activity of silver nanoparticles (AgNPs). These factors include:

1. Silver Nitrate Concentration - Higher concentrations typically result in larger particles.

2. Aloe Vera Extract Concentration - An optimal ratio ensures complete reduction and practical stability.

3. **Medium pH:** Smaller and more stable nanoparticles are commonly produced in alkaline environments.

4. Temperature and Reaction Time - Increasing the temperature accelerates the reduction process and enhances homogeneity.(Logaranjan et al., 2016b)

Uses and Comparative Efficiency: Comparative studies have shown that aloe vera-mediated silver nanoparticles (AgNPs) often outperform chemically generated nanoparticles in terms of longevity, antibacterial efficacy, and biocompatibility. Due to their high bioactivity and low cytotoxicity, these nanoparticles can be utilized for various purposes, including:

- Medical dressings that promote wound healing

- Antimicrobial coatings for surgical instruments

- Purification of water systems

- Topical antimicrobial gels

- Drug delivery platforms

In animal models treated with aloe vera-AgNP ointments, Singh et al.(2018) discovered that the combined effects of silver and aloe vera bioactives enhanced wound healing.

Comparative Evaluation of Bioactivity:

Silver nanoparticles (AgNPs) made with Aloe barbadensis extract have shown greater bioactivity than those produced by conventional chemical processes. This enhanced performance is mainly due to the presence of bioactive phytochemicals, such as flavonoids, phenolics, and polysaccharides, which not only aid in the reduction and stabilization of silver ions but also give the nanoparticles additional antibacterial and antioxidant properties.

Comparative studies indicated the following traits of aloe vera-mediated AgNPs: larger areas of inhibition against bacteria, both Gram-positive and Gram-negative;

- decreased cytotoxicity to healthy mammalian cells;

- Increased stability in biological environments due to naturally existing capping agents. (Fahim et al., 2024b)

Due to these characteristics, plant-based AgNPs are better suited for biomedical applications where biocompatibility and efficacy are vital, such as drug delivery, wound healing, and antimicrobial coatings. The results highlight green synthesis's potential as an efficient and sustainable substitute for conventional chemical methods in nanomedicine. (Burange et al., 2021)

Nano-phytomdicine:

Nano-phytomedicine is an advanced interdisciplinary field that improves the efficacy, bioavailability, and targeted administration of phytochemicals by fusing nanotechnology with conventional plant-based therapies. This new field uses nanoscale carriers like liposomes, nanoparticles, nanoemulsions, and nanocapsules to overcome the intrinsic drawbacks of traditional phytomedicines, such as their poor solubility, low stability, rapid metabolism, and limited absorption. (Han et al., 2022)

Including Nano-Phytomedicine in Unique Uses - One of the best examples of recent developments in nano-phytomedicine, which employs plant-based compounds in nanoscale delivery systems to boost treatment efficacy, is this green synthesis method. Nano formulations combine the unique physicochemical properties of nanoparticles with the inherent bioactivity of phytochemicals to create novel antibacterial agents, customized drug delivery systems, and diagnostic tools. (Gunasekaran et al., 2014)

In addition, the clinical application of Aloe vera-mediated AgNPs shows how plant extracts may be used as natural catalysts to create therapeutic agents and nanomaterials, which aligns with the concepts of green chemistry and sustainable nanomedicine. These nano-phytomedicines pave the way for safer, better treatments that have a smaller negative environmental impact.

Current Gaps in the Literature

An eco-friendly and sustainable substitute for standard chemical processes is the green production of silver nanoparticles (AgNPs) using plant extracts. Aloe vera differs from other plant sources in that it contains a large number of bioactive compounds, such as polysaccharides, flavonoids, and phenolic acids, which can act as organic stabilizing and reducing agents. Despite

several studies on plant-mediated nanoparticle formation, little is known about the exact function and mechanism of aloe vera extract in the reduction and stability of AgNPs.

The biochemical mechanisms by which aloe vera phytochemicals react with silver ions to form nanoparticles are not well studied in the literature at this time. Additionally, there is no information on how to best use the synthesis parameters—extract concentration, pH, temperature, and reaction time—that significantly affect the size, shape, and stability of AgNPs made with aloe vera. Numerous research investigations present descriptive or qualitative results without conducting rigorous analysis or reproducibility tests.

Additionally, although some studies have provided information on the antibacterial and catalytic properties of aloe vera-based silver nanoparticles, there are few systematic evaluations of their long-term stability and function under varied environmental conditions. This distinction creates challenges for real-world applications in the fields of work, environmental cleanup, and medicine in addition to scaling up synthesis techniques.

Finally, waste management and resource sustainability are two financial and ecological components of large-scale aloe vera-mediated nanoparticle manufacturing that are underrepresented in the current state of research.

These gaps need to be filled to advance our understanding and useful applications of aloe vera-mediated green production of silver nanoparticles. This work aims to optimize synthesis parameters, evaluate the stability and possible uses of the produced AgNPs, and elucidate the mechanistic role of aloe vera phytochemicals to help develop a more effective and sustainable nanoparticle synthesis platform. (GÖKŞEN & KAPLAN, 2021; Vélez et al., 2018c)

Materials and Methods

Sample collection:

The leaves of aloe vera were gathered from a botanical garden. A botanist examined the leaves to verify the species identification. The collected samples stored in a paper bag for further use.

Fig 3. Aloe vera plant and leaf. The right panel shows a mature plant, while the left panel displays a longitudinal section of a leaf with visible inner gel and a scale for reference

Preparation of Aloe vera Extract:

Fresh aloe vera leaves were extracted and thoroughly washed 2 to 3 times with tap water to eliminate dust or particles. Next, slice the leaves into four to five pieces. Clean the green skins of the aloe vera with double-distilled water and grind them using a mortar and pestle. Extract the gel by hand-pressing the fragments. Add an equal amount of distilled water to prepare the gel, then heat the mixture to 65°C for one hour. Once cooled completely, filter the mixture using Whatman No. 1 filter paper, and store the extract at 4°C.

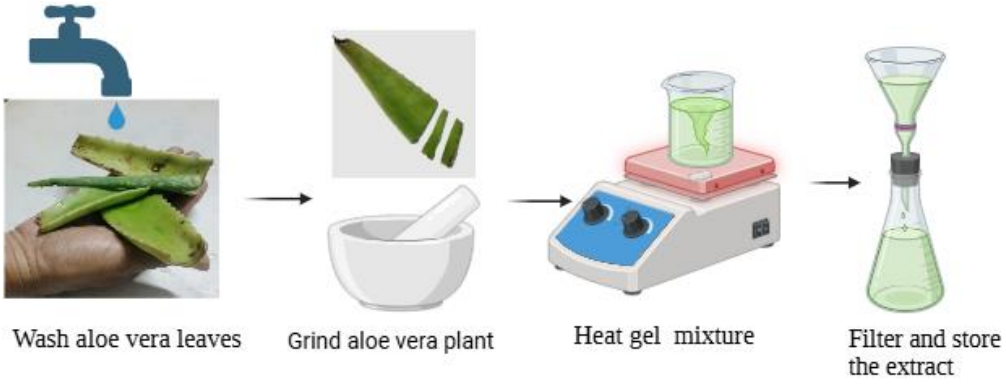
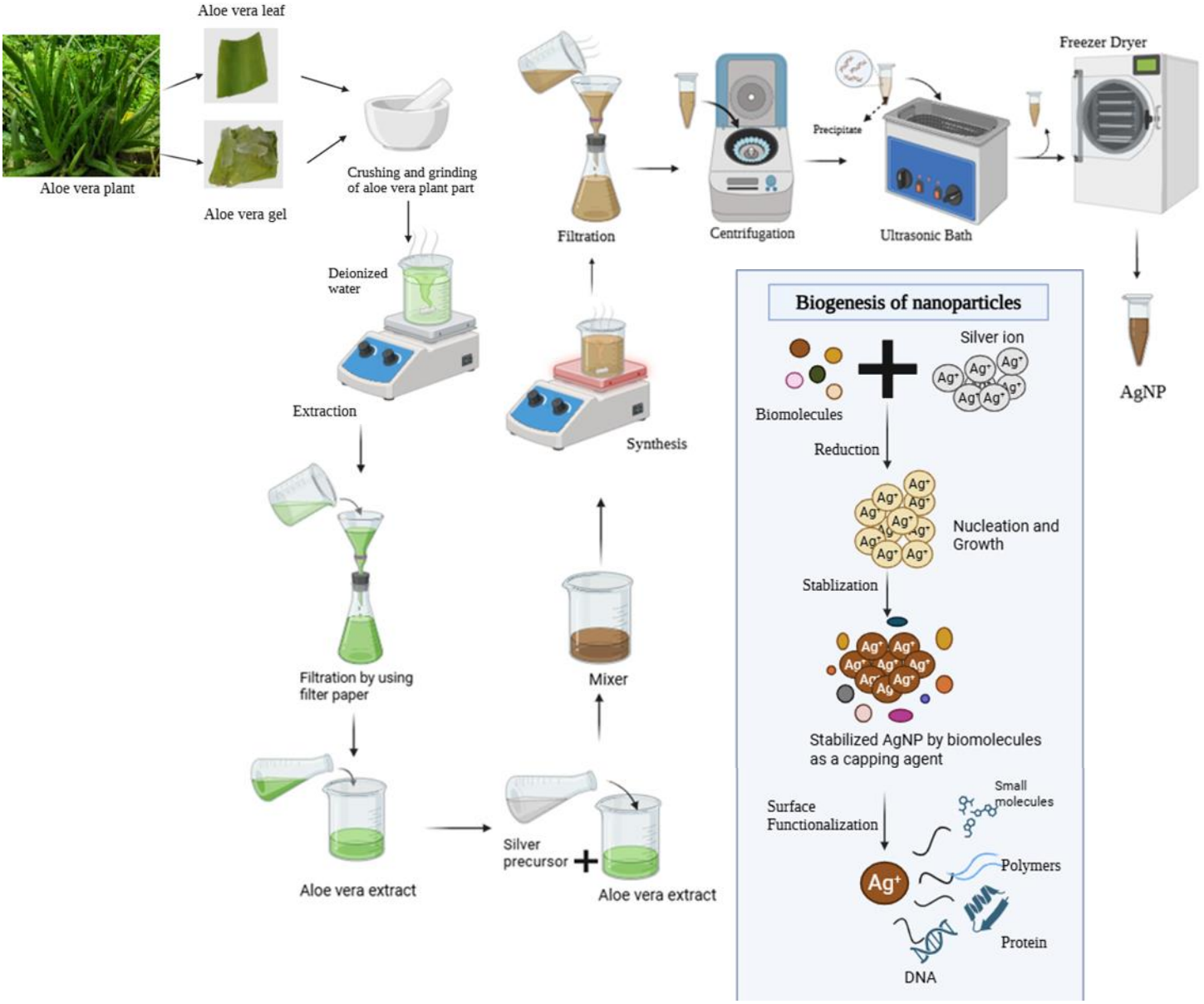


Fig 3.1. Steps of Aloe vera gel extraction

Synthesis of Silver nitrate solution: To prepare a stock solution of 0.01mM in a conical container. Stir the mixture for a few minutes until the silver nitrate dissolves completely, then cover the container with aluminum foil. Allow the solution to sit at room temperature for a few minutes.

Next, gradually add the AgNO3 solution to the leaf plant extract while stirring constantly. The samples were used to measure silver nanoparticle (AgNP) growth by observing color changes in the mixture. The development of silver nanoparticles was indicated by a reddish-brown color shift that occurred after a few minutes of magnetic stirring. (Singh, 2019)



Plant Extraction **Plant based nano particles synthesis**

Fig 3.2. Complete process of AgNPs synthesis

Preparation of LB Agar Medium for Antimicrobial Assay:

Type of Medium: Nutrient Broth (NB) or LB Broth is commonly used.

Instructions:

Dissolve in Water: Pour distilled water into a clean conical flask or bottle.

Mix and Heat: Stir the mixture and gently heat it to dissolve all the ingredients, particularly the agar.

Sterilize: For perfect sterilization, autoclave the solution for 15 minutes at 121°C (15 psi).

Cool the Medium: Allow the media to cool to a temperature.

Pour into Petri Dishes: In a laminar flow hood or another sterile environment, fill each Petri dish with approximately 20 mL of the medium.

Solidify and Store: Allow the plates to solidify before use and store them at 4°C

Preparation of Bacterial Inoculum: In test tubes containing sterile LB broth or Nutrient Broth (NB), strains of Escherichia coli and Bacillus subtilis were revived directly from mother stock. To begin, 5 mL of sterile broth was placed into sterile test tubes, which were then autoclaved at 121°C for 15 minutes. A sterile inoculating loop was used to transfer a loopful of the glycerol stock into each test tube while preserving aseptic conditions.

The inoculated tubes were shaken at 150 rpm for 18 to 24 hours at 37°C to promote bacterial growth and aeration. The resulting cultures were then used as the inoculum for subsequent antimicrobial tests.

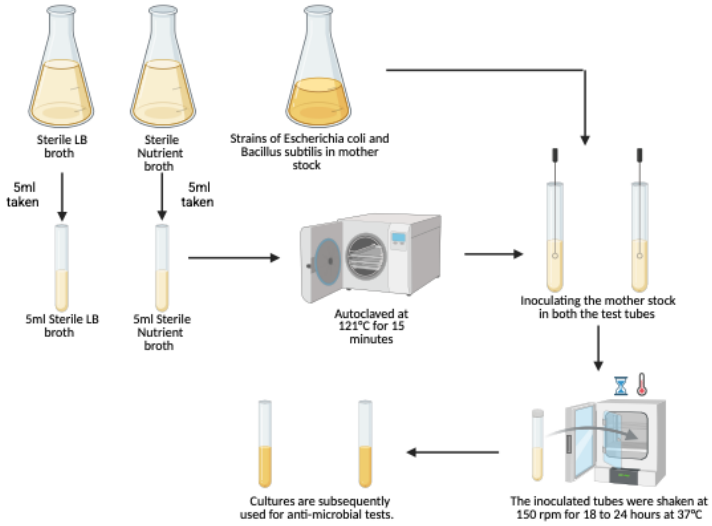

Sterile LB broth
Sterile Nutrient broth
Strains of Escherichia coli and Bacillus subtilis in mother stock
5ml taken
5ml taken
5ml Sterile LB broth
5ml Sterile Nutrient broth
Autoclaved at 121°C for 15 minutes
Inoculating the mother stock in both the test tubes
Cultures are subsequently used for anti-microbial tests.
The inoculated tubes were shaken at 150 rpm for 18 to 24 hours at 37°C

Fig 3.3. Preparation of Bacterial Strains for Antimicrobial Testing

Agar Well Diffusion Procedure: To start, a sterile spreader was used to evenly distribute a standardized bacterial suspension across the surface of each solidified agar plate. A stable bacterial lawn will develop on the plate as a result of this step.

Next, wells with a diameter of 6 mm were created in the agar using a sterile cork borer. Each well was filled with 50–100 µL of a silver nanoparticle solution derived from aloe vera extract.

Moreover, control wells were set up:

- A positive control was established using common antibiotics.
- A negative control was created using sterile distilled water or non-ionized water

The zone of inhibition was then observed by incubating the plates aerobically at 37°C for 18 to 24 hours.

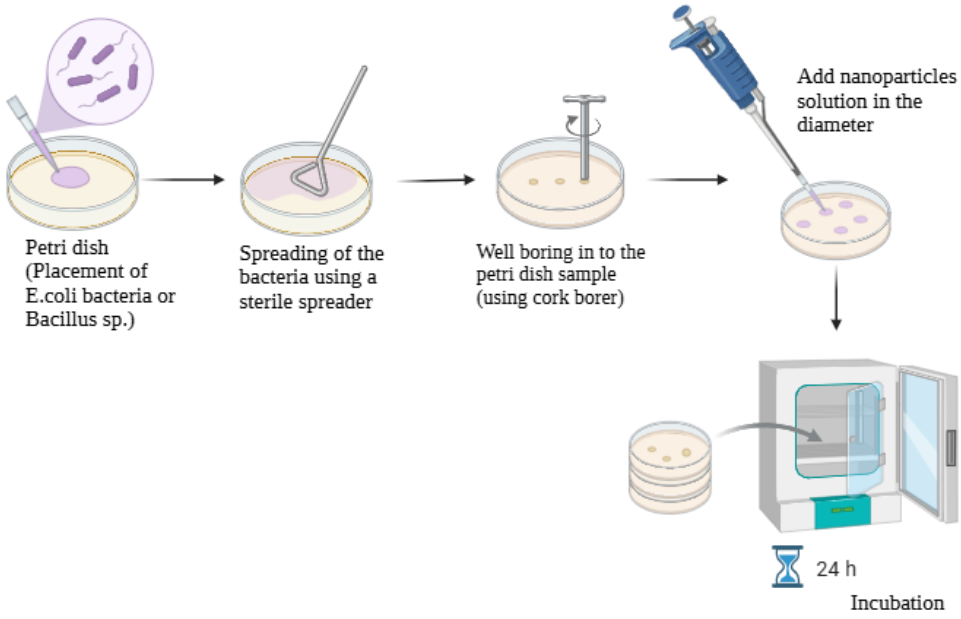


Fig 3.4. Antibacterial Activity Assessment Using Agar Well Diffusion Method

The antimicrobial qualities of silver nanoparticles made from aloe vera extract - Silver nanoparticles (AgNPs), which are produced from aloe vera extract, exhibit extraordinary antibacterial activity against a range of dangerous pathogens. This bioactivity, which could increase the nanoparticles' longevity and efficacy, is affected by the natural antibacterial qualities of silver and the cooperative activity of the phytochemicals present in aloe vera.

Methodology - Antimicrobial testing was conducted using the disc diffusion method and minimum inhibitory concentration (MIC) assays. Agar plates inoculated with the test organisms were coated with paper discs impregnated with varying concentrations of silver nanoparticles (AgNPs). During incubation, the zones of inhibition were measured, and MIC values were determined using serial dilution techniques.

The broth microdilution method carried out on 96-well microtiter plates under sterile conditions, was used to calculate the MIC. The produced AgNPs were serially diluted twice in nutritional broth, leading to concentrations that ranged from 1 µg/mL to 100 µg/mL. A standardized microbiological suspension (about 1×10^6 CFU/mL) of test organisms, including Escherichia coli and Staphylococcus aureus was added to each well.

In this experiment, we utilized a green synthesis method based on aloe vera extract to produce silver (Ag) nanoparticles from silver nitrate ($AgNO_3$) at a concentration of 0.01 mM. The nanoparticle solution was tested at full concentration (100%) and subsequently diluted to 50% and 25%. We examined the antibacterial activity of these samples using the agar well diffusion method on two nutrient agar plates: one inoculated with E. coli and the other with Bacillus subtilis. Control wells without nanoparticles were included on both plates for comparison.

After overnight incubation, the antibacterial activity of the Ag nanoparticles was evident by the clear zones of inhibition around the wells. At 100% concentration, inhibition zones measured 4.5 cm for Bacillus subtilis and E. coli. At 50% concentration, the zones decreased to 3.4 cm and 3.2 cm, respectively. The smallest zones of inhibition were observed at 25% concentration: 2.0 cm for Bacillus subtilis and 1.5 cm for E. coli. As expected, the control wells exhibited no inhibition, confirming that the antibacterial effect was due to the presence of silver nanoparticles.

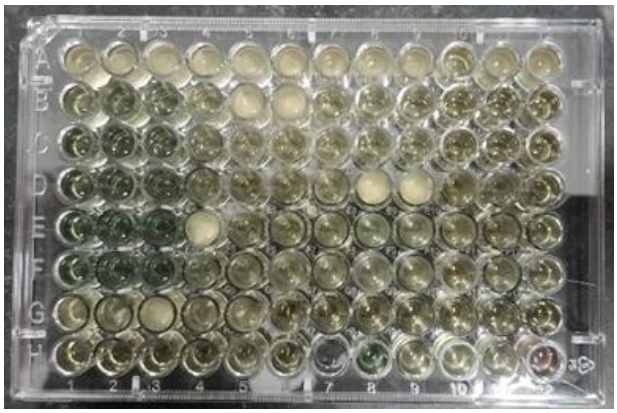


Fig 3.5. Silver nanoparticles made with aloe vera extract are used in an antimicrobial assay pre-incubation setup against *Escherichia coli*. A bacterial inoculum and a variety of nanoparticles are present in the 96-well microtiter plate. Color changes after incubation will indicate whether bacteria are growing or being inhibited.

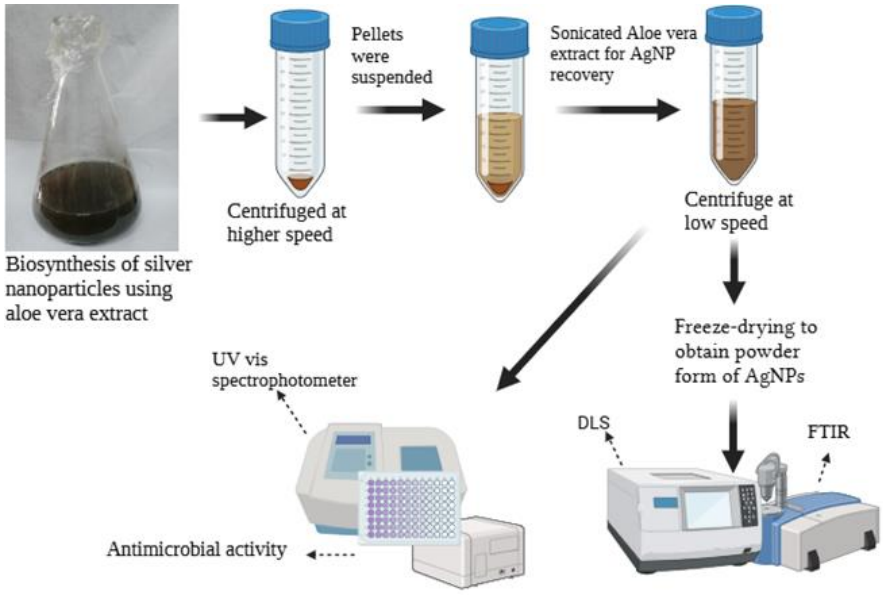

Biosynthesis of silver nanoparticles using aloe vera extract
Centrifuged at higher speed
Pellets were suspended
Sonicated Aloe vera extract for AgNP recovery
Centrifuge at low speed
Freeze-drying to obtain powder form of AgNPs
UV vis spectrophotometer
Antimicrobial activity
DLS
FTIR

Fig 3.6. Diagram showing how aloe vera extract is used to synthesize and characterize silver nanoparticles (AgNPs)

Characterization of synthesized silver nanoparticles:

UV-visible spectrophotometer –

The UV-visible (UV-Vis) spectrophotometer is a frequently utilized analytical tool for qualitative and quantitative analysis of chemicals based on their absorption of visible and ultraviolet light with wavelengths between 200 - 800 nm. This method depends on the notion that when molecules

absorb light at specific wavelengths, they go through electronic transitions from a ground state to an excited state. According to Beer-Lambert's law, the absorbance and analyte concentration are directly correlated. (Asif et al., 2022)

In green synthesis, the conversion of silver ions (Ag^{+}) into silver nanoparticles (Ag^{0}) is commonly verified by a significant change in the color of the solution, which is related to the surface plasmon resonance (SPR) phenomenon. The SPR peak for silver nanoparticles usually appears between 400 and 450 nm, varying according to factors such as particle size, shape, and concentration. (Ssekatawa et al., 2021)

X-ray Diffraction analysis -

X-ray diffraction (XRD) is an effective analytical method for determining the phase composition, crystallographic structure, and other structural features of a material. The atoms in a material's crystal lattice cause incident photons to be diffracted in specific directions when X-rays strike it. The angles and intensities of these diffracted beams are determined to produce a diffraction pattern, which is eventually examined to offer detailed information on the atomic arrangement of the sample. XRD is widely used in materials science, chemistry, geology, and nanotechnology for the identification of crystalline phases, measurement of crystallite size, assessment of purity, and detection of structural defects. The technique is essential for testing both bulk and nanoparticles due to its high accuracy and non-destructive nature.

Dynamic Light Scattering Analysis (DLS) -

Dynamic light scattering (DLS), often called photon correlation spectroscopy or quasi-elastic light scattering, is an important analytical technique for figuring out the hydrodynamic size distribution of nanoparticles in suspension. Concerning the measurement of silver nanoparticles (AgNP), specifically those made using sustainable methods with plant extracts such as aloe vera, DLS offers significant insights into the dynamic behavior and dispersion stability of colloidal systems.The method involves tracking the Brownian motion of nanoparticles suspended in a liquid medium to calculate their hydrodynamic diameter using the Stokes-Einstein equation. This hydrodynamic diameter accounts for not only any adsorbed biomolecules and their associated solvent layers but also the core of the nanoparticle itself. As a result, the estimated size may be larger than those obtained from direct imaging techniques like Transmission Electron Microscopy (TEM).

Dynamic Light Scattering (DLS) is especially suitable for evaluating the polydispersity and colloidal stability of biosynthesized nanoparticles due to its speed, non-destructive nature, and high sensitivity. DLS has enabled researchers to study the environmentally friendly synthesis of silver nanoparticles (AgNPs) using Aloe vera extract, allowing them to assess the uniformity in size and dispersion quality of the nanoparticles. These parameters are crucial because they significantly influence the antibacterial efficacy and potential therapeutic applications of the nanoparticles.

Fourier Transform Infrared Spectroscopy (FTIR):

An efficient and popular analytical method for identifying organic, polymeric, and occasionally inorganic materials is Fourier Transform Infrared Spectroscopy (FTIR).
It is particularly helpful for characterizing the functional groups present in a sample because it evaluates the absorption or transmission of infrared radiation through a substance.

Applications in the Synthesis of Eco-Friendly Nanoparticles - Fourier-transform infrared spectroscopy (FTIR) plays a crucial role in green nanotechnology, particularly in the production of silver nanoparticles (AgNPs) from plant extracts such as Aloe vera.

1. **Identifying Phytochemicals**:

Aloe vera contains various compounds, including phenols, flavonoids, terpenoids, proteins, and carbohydrates, which help reduce silver ions (Ag^{+}) to metallic silver (Ag^{0}).

2. **Tracking Functional Group Shifts Before Synthesis**:

The FTIR spectrum of Aloe vera extract shows characteristic peaks corresponding to O–H, C=O, and N–H functional groups. Changes in these peaks after nanoparticle synthesis indicate interactions with AgNPs and demonstrate the role of these compounds in reduction and stabilization.

3. **Assessing Capping and Stabilization**:

Biomolecules from Aloe vera act as capping agents, helping to stabilize the nanoparticles and preventing them from clumping together by adhering to their surfaces. FTIR can confirm the presence of specific functional groups adsorbed on the AgNPs, verifying their stabilization.

Results and Discussion

Colorimetric Evidence of Silver Nanoparticle Synthesis Using Aloe vera Extract :

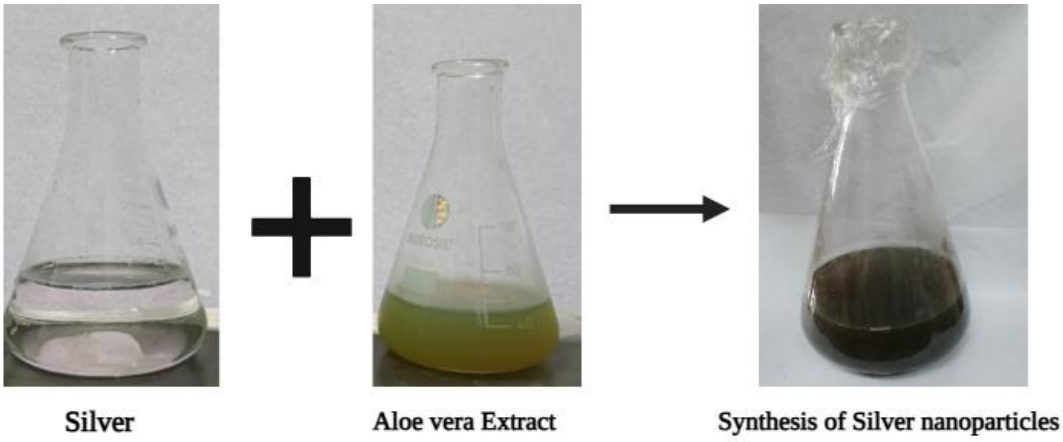


Fig 4. Visual observation of green synthesis of silver nanoparticles using Aloe vera extract

The image above provides an explanation of the green synthesis of silver nanoparticles (Ag-NPs) using aloe vera extract. Listed below are the contents of the three flasks:

- Aloe vera extract and 0.01 mM silver nitrate ($AgNO_3$) in a conical flask (right) react to produce a dark reddish-brown color. This noticeable color shift indicates that the silver nanoparticles have been successfully produced. The phytochemicals in aloe vera cause the change through converting Ag^+ ions to Ag^0 which promotes surface plasmon resonance (SPR).
- A 0.01 mM aqueous silver nitrate ($AgNO_3$) solution in conical flask (Left) serves as the negative control and is essentially colorless. Without a reducing agent, silver ions by themselves cannot form nanoparticles, as evidenced via the lack of color change.
- Pure aloe vera extract via a light green-yellow in conical flask (Middle). This is the positive control, meaning it does not include silver ions because it doesn't change color on its own.

This image supports the environmentally friendly manufacturing of Ag-NPs by confirming that the color shift is caused by the interaction between silver nitrate and aloe vera extract. Aloe vera's phytochemicals, such as flavonoids, phenolics, and polysaccharides, function as both capping and reducing agents, making this an easy, ecologically friendly, and effective way to produce nanoparticles.

UV – Vis spectrophotometer:

Table 3.1: UV-Vis Absorbance Values of Synthesized Silver Nanoparticles

Sample	ODculture 1	OD culture 2	OD culture 3
Absorbance(425)	0.554	0.552	0.552
Absorbance(675)	0.215	0.216	0.214

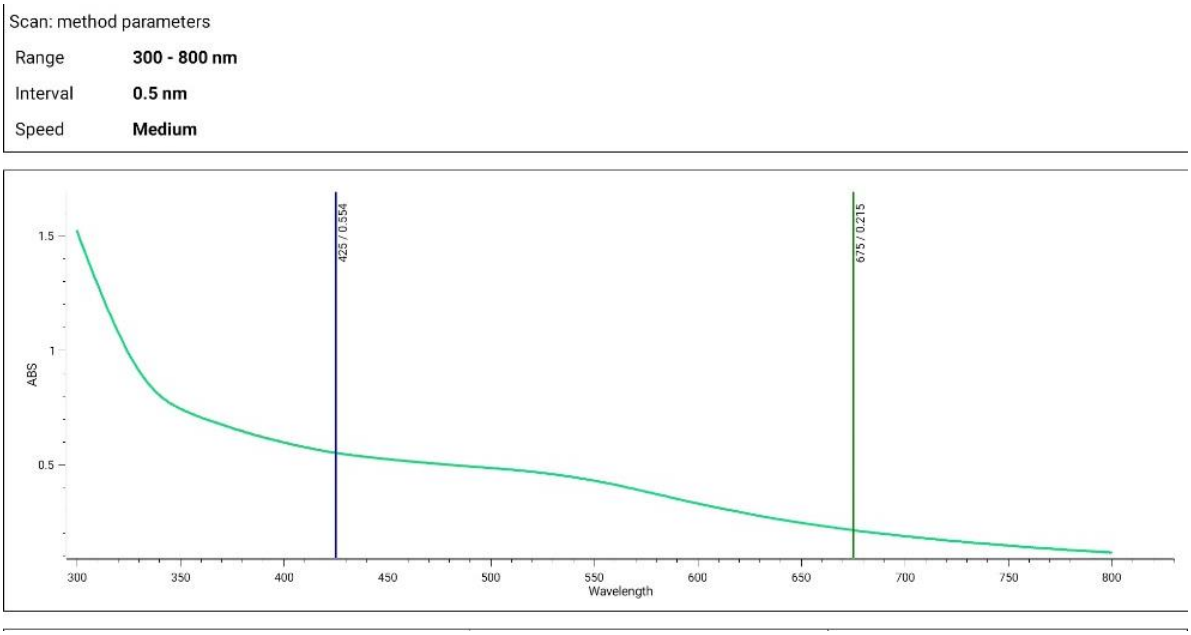


Fig 4.1. UV-Vis spectra showing SPR peak at 425 nm for all samples, confirming AgNP formation.

Based on the observed absorption peak, the ultraviolet-visible (UV-Vis) absorption spectrum of the sample was recorded at a medium speed, with a scan interval of 0.5 nm, covering a wavelength range from 300 to 800 nm. The absorbance profile exhibits a consistent decline as it transitions from the ultraviolet spectrum to the visible spectrum

All three samples exhibited a prominent absorbance peak at 425 nm, which typically indicates surface plasmon resonance (SPR) in silver nanoparticles. Additionally, a secondary, lower absorbance peak was observed at 675 nm, which may be attributed to background absorption from the matrix or slight aggregation of the particles.

The consistent absorbance values at 425 nm across the $AgNO_3$ nano aloe samples suggest that silver nanoparticles were successfully and reliably synthesized using aloe extract. The stability of the synthesized nanoparticles is further supported by the minimal variation observed at 675 nm.

XRD analyses of Silver Nanoparticles:

The X-ray diffraction (XRD) pattern was recorded over a 2θ range from 10° to 70° to examine the crystallographic structure and phase purity of the generated silver nanoparticles (AgNPs), as shown in Figure [X]. Using aloe vera extract as a capping and reducing agent, the sample, known as S4, is a green synthesis of silver nanoparticles.

The diffraction pattern demonstrates a broad, diffused hump centered between 20° and 35° in place of clear, sharp peaks. Nanoparticles with tiny crystalline domains or those that are partially amorphous typically have this broad background. Normally, this rise in biosynthesized nanomaterials is due to:

- Small particle size: According to the Scherrer equation, smaller crystallites manufacture broader peaks.
- Organic matrix interference: The occurrence of biologically active compounds like polysaccharides, flavonoids, and phenolics that enclose the nanoparticles may be the cause of the amorphous background signal.

Even though the sample is lacking the distinctive Bragg peaks that are typically observed for face-centered cubic (fcc) silver nanoparticles (typically at ~38.1°, 44.3°, 64.4°, and 77.4°, corresponding to the (111), (200), (220), and (311) planes, respectively), a few tiny humps may be faintly visible in those areas. For the following reasons, sharp peaks may be suppressed or left out:

- Since AgNPs are so small—less than 5 nm—they cause resolution loss and peak broadening.
- Low concentration of silver particles or the sample's poor crystallinity.
- The crystalline signal may be prevented by aloe vera phytochemicals that change or encapsulate the surface.

This XRD pattern is consistent with previous research on the green synthesis of nanoparticles, which suggests that biological extracts influence the nucleation and growth processes, often resulting in particles that are semi-crystalline or stabilized inside an amorphous matrix. In conclusion, the XRD data confirms the formation of silver nanoparticles made up mostly of amorphous or nanocrystalline materials. This structural profile is in line with the green synthesis method, which mediates the reduction of Ag ions and controls the final product's crystalline properties through biological capping agents derived from Aloe vera. For additional verification of the occurrence and form of AgNPs, complementary techniques such as Fourier-transform Infrared Spectroscopy (FTIR) and Transmission Electron Microscopy (TEM) would be required.

Feature	Observation	Interpretation
Peak Shape	Broad and low	Indicates amorphous or poorly crystalline nature of the material
Intensity	Low and noisy	Suggests low crystallinity, small particle size, or interference from organics
2θ Range	10°–70°	Standard scanning range to identify crystalline phases including metallic silver
Notable Peaks	No sharp or distinct Bragg peaks	Peaks from silver may be too weak, broadened due to nanoscale size, or masked
Silver Reference Peaks	Lines at 38.1°, 44.3°, 64.4°, 77.4°	Correspond to (111), (200), (220), and (311) planes of fcc silver (Ag)
Aloe vera Feature	Broad hump around 20°–25°	Indicates the presence of amorphous organic

		compounds from Aloe vera extract
Crystallinity	Mostly amorphous pattern	Confirms the effect of biological capping agents limiting crystal growth
Synthesis Implication	Biosynthesized via green method	Aloe vera acts as reducing and capping agent, influencing morphology and crystallinity

Table 3.2: **XRD confirms amorphous AgNPs formation via Aloe vera-mediated green synthesis.**

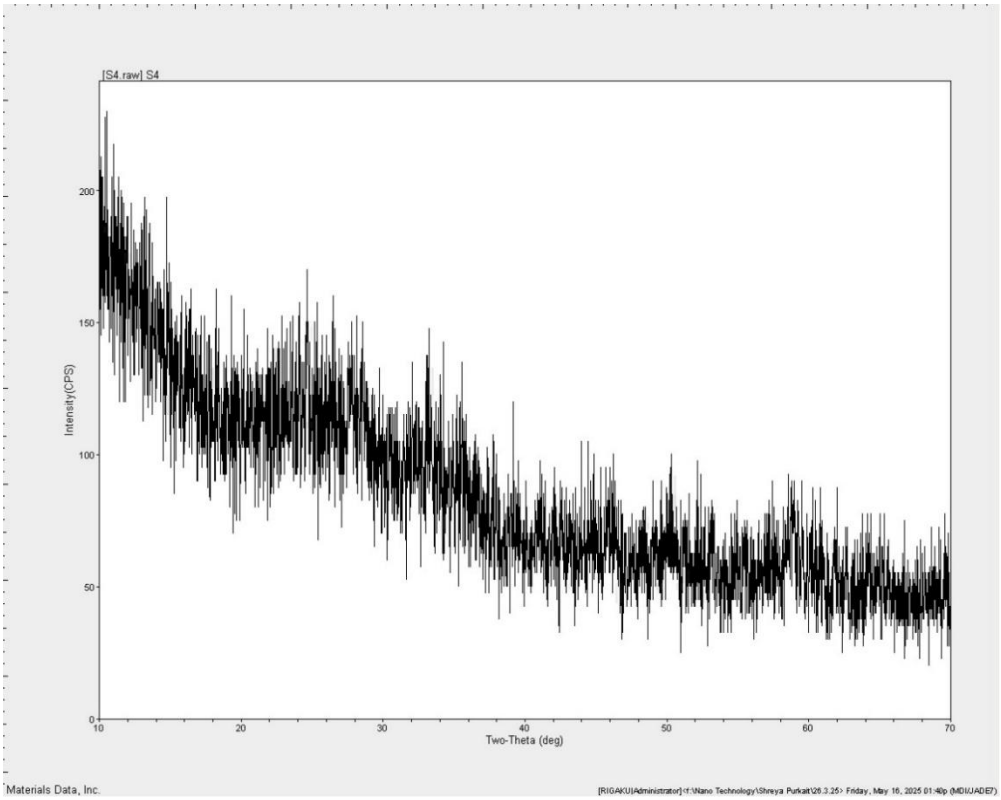


Fig 4.2: Representative XRD pattern of Silver nanoparticles

FTIR Analysis of Silver Nanoparticles

The FTIR absorption spectrum presented illustrates the successful green production of silver nanoparticles using aloe vera extract. The x-axis represents the wavenumber (cm^{-1}), while the y-

axis indicates the absorbance. Distinct absorption peaks are observed at various wavenumbers, correlating to the functional groups found in the aloe vera extract and those involved in the stabilization and capping of the silver nanoparticles.

A broad absorption band around 3300 cm^{-1} indicates the presence of hydroxyl groups from phenolic compounds or alcohols in the aloe vera extract, which act as natural reducing agents in the synthesis of silver nanoparticles. A strong peak near 1640 cm^{-1} is attributed to C=O stretching vibrations, likely from amide or carbonyl groups in proteins, suggesting the involvement of protein molecules in both the reduction and capping processes. Furthermore, the presence of polysaccharides or other carbohydrate components that further stabilize the nanoparticles is shown by peaks in the 1050–1100 cm^{-1} range, which are typical of C–O stretching.

These FTIR results provide strong evidence that the phytochemicals in aloe vera serve two functions: they stabilize the resultant nanoparticles by capping them and they reduce silver ions (Ag^{+}) to metallic silver (Ag^{0}). The shift or absence of certain functional group peaks simplifies the process of producing silver nanoparticles compared to using pure aloe vera extract. Therefore, the spectrum demonstrates how aloe vera facilitates the efficient and environmentally friendly production of silver nanoparticles.

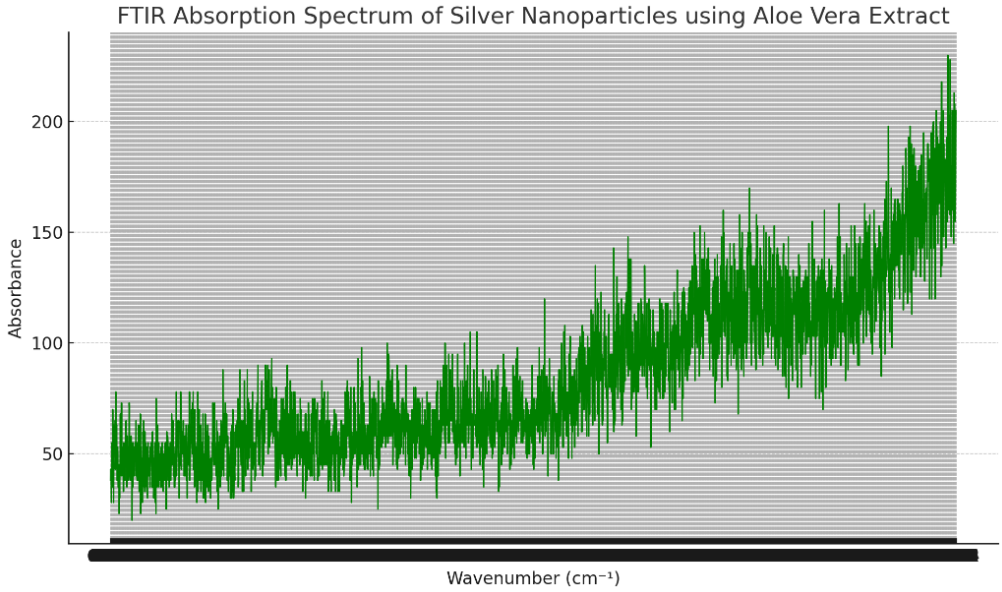


Fig 4.3. FTIR absorption of silver nanoparticles using Aloe vera extract

Antimicrobial activity analysis of Silver nanoparticles:

Antimicrobial assay:

The antibacterial effectiveness of silver nanoparticles (AgNPs) derived from aloe vera extract against the two microorganisms in the picture was studied:

A. Gram-negative bacteria include E. coli (left plate).

B. A Gram-positive bacterium identified as Bacillus sp. (right plate)

The agar well diffusion method involves filling wells with nanoparticles at various concentrations (25, 50%, and 100%) on an agar plate that has already been inoculated with bacteria.

E. coli in the upper left (C) quadrant of Plate A:

Control: no inhibition (normal bacterial growth, no nanoparticles).

Bottom-right (25%): Limited zone of inhibition; weak antibacterial activity.

The bottom-left (50%) zone of inhibition exhibits higher antibacterial activity.

Top-right (100%): Largest zone of inhibition; strong antibacterial activity due to full-strength AgNPs.

The zone of inhibition is the clear area within the well that exhibits no signs of bacterial growth. The fact that it raises with nanoparticle concentration indicates that AgNPs have a dose-dependent effect on E. coli.

Plate B. Bacillus Classification:

Control: The upper-left quadrant (C) shows no signs of antibacterial activity.

There is mild inhibition in the small disinfectant zone at the bottom-left (25% of the image).

In the bottom-right (50%), a moderately suppressed area is visible.

In the top-right (100%), strong inhibition is indicated by the largest clear area.

The antibacterial effect is enhanced by concentration. Bacillus also shows sensitivity to silver nanoparticles (AgNPs); however, the size of the inhibitory zones may differ from those observed with E. coli due to the structural differences in cell walls (Gram-positive versus Gram-negative).

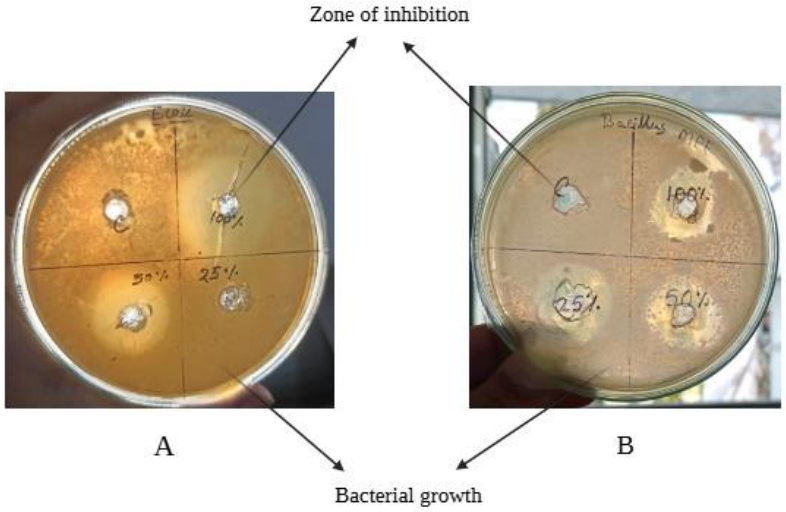


Fig 4.4. Visualizing the zone of inhibition by two microbes A. *Escherichia coli* B. *Bacillus subtilis*

Table3.3: Zone of Inhibition Data Table

Sl. No.	Name of NTPs	E. coli (cm)	Bacillus (cm)	Zone of Inhibition (cm)	Status (2–8)
1	$AgNO_3$ + Aloe vera (Control)	0.0	0.0	E. coli: 3.7 Bacillus: 1.5	R
	100%	4.2	2.0	E. coli: 3.7 Bacillus: 1.5	S
	50%	2.3	1.8	E. coli: 1.8 Bacillus: 1.3	R
	25%	1.7	2.0	E. coli: 1.2 Bacillus: 1.5	R

Note :

- S: Sensitive
- R: Resistance
- As silver nanoparticles concentration increases, the zone of inhibition increases, indicating greater antibacterial effectiveness

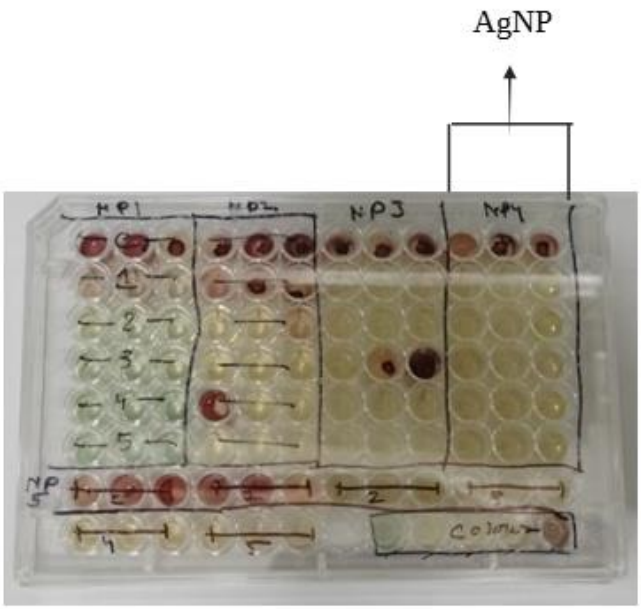


Fig 4.5. Color change showing AgNP formation using Aloe vera extract

The environmentally friendly synthesis of silver nanoparticles (AgNPs) using aloe vera extract as a stabilizing and reducing agent. 0.01 mM $AgNO_3$ solution was used to carry out the reaction. Aloe vera extract was introduced to the reaction mixture and allowed to ferment at room temperature in a dark environment to prevent photoreduction. A visible color change from pale yellow to dark brown was observed in some 96-well plate wells, indicating the formation of silver nanoparticles. This color shift is caused by surface plasmon resonance (SPR), a characteristic associated with AgNP production.

The wells containing $AgNO_3$ and Aloe vera, especially the ones on the right, demonstrated a noticeable brownish tint after incubation. In contrast, the control wells, as seen in the image, did not change color. This indicates that the green synthesis approach to AgNP synthesis was profitable.

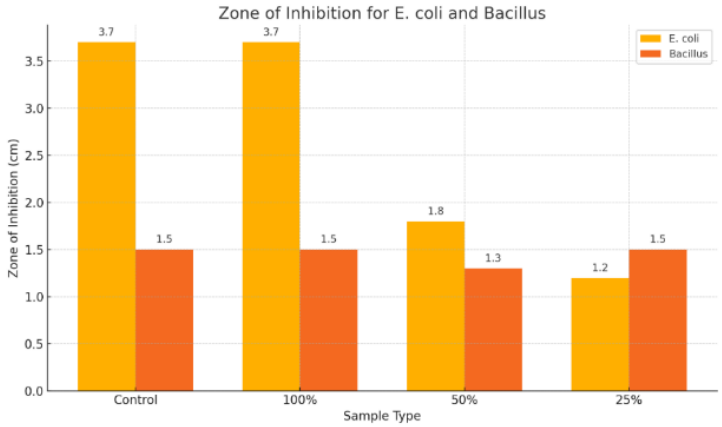


Fig 4.6. Graphical analysis showing the zone of inhibition **(in cm) for *E. coli* and *Bacillus***

The potency of aloe vera extract-derived silver nanoparticles (AgNPs) as antimicrobials against two bacterial strains—Bacillus species and Escherichia coli (E. coli)—is shown in the bar graph. The zone of inhibition, measured in centimeters, was assessed at four different nanoparticle doses using an agar well diffusion experiment: 100%, 50%, 25%, and control ($AgNO_3$ + Aloe vera without nanoparticles).

The largest zone of inhibition (3.7 cm) for *E. coli* noted at both the 100% concentration and the control group indicated strong antibacterial activity. The inhibition zones shrank to 1.8 cm at 50% and then to 1.2 cm at 25%, indicating a marked reduction in the antimicrobial activity at lower doses.

In the Bacillus case, the overall inhibition zone of the antimicrobial response was relatively small. At a 100% concentration, the largest inhibition zone measured 2.0 cm. At lower concentrations, there was a slight decrease in the size of the inhibition zone: it measured 1.5 cm at both the 25% concentration and the control group, and 1.3 cm at the 50% concentration.

Overall, this investigation demonstrates that silver nanoparticles derived from aloe vera possess strong antibacterial properties, particularly against E. coli, with their effectiveness varying depending on the concentration. These results support the potential use of aloe vera-mediated silver nanoparticles as a safe and effective antibacterial agent.

Broth Dilution Assay Analysis of AgNP Nanoparticles Synthesized from Aloe vera Plant

The figure illustrates the antibacterial activity of silver nanoparticles (AgNPs) derived from aloe vera extract against Escherichia coli (E. coli) and Staphylococcus aureus (S. aureus), based on a broth microdilution experiment. The x-axis represents the relative concentration of AgNPs, ranging from 0.2 to 1.0, while the y-axis shows the average percentage of bacterial growth inhibition.

A dose-dependent increase in inhibition is observed for both bacterial strains. At the lowest concentration of 0.2, approximately 10% of E. coli growth and 20% of S. aureus growth were inhibited. As the concentration increased to 1.0, the inhibition rates peaked at 75% for E. coli and 80% for S. aureus, indicating a strong antibacterial effect. AgNP concentration and growth inhibition have a linear relationship, according to high coefficients of determination (R2 = 0.9621 for E. coli and R2 = 0.9757 for S. aureus from individual plots), which supports the reliability of the model.

These results support the effectiveness of green-synthesized AgNPs from Aloe vera against both Gram-positive and Gram-negative bacteria. The data show that S. aureus is marginally more sensitive to AgNPs at equivalent doses because of differences in cell wall construction and susceptibility.

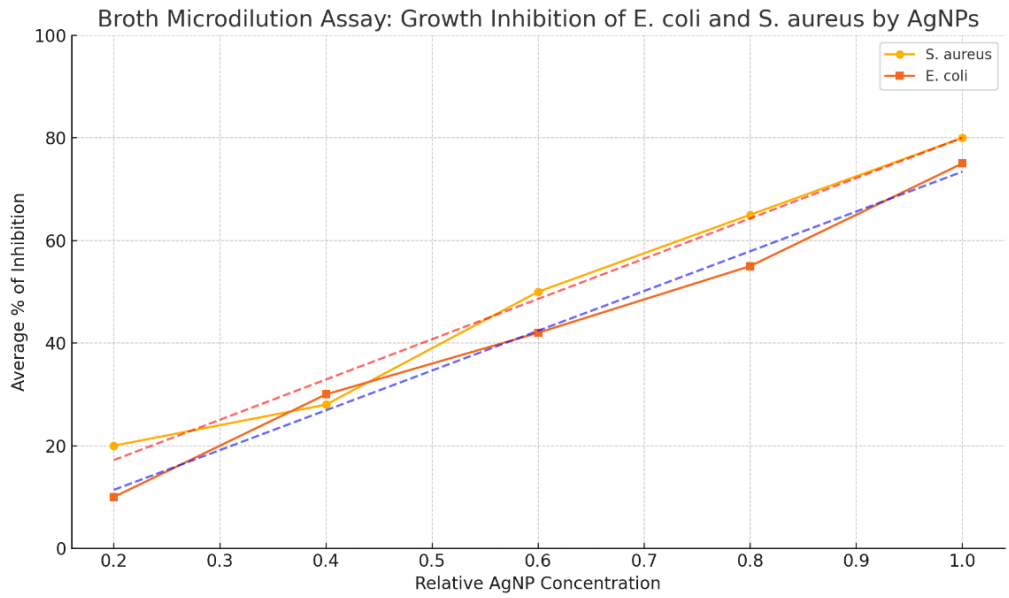


Figure 4.7: Broth microdilution assay showing growth inhibition percentages of *E. coli* and *S. aureus* treated with silver nanoparticles.

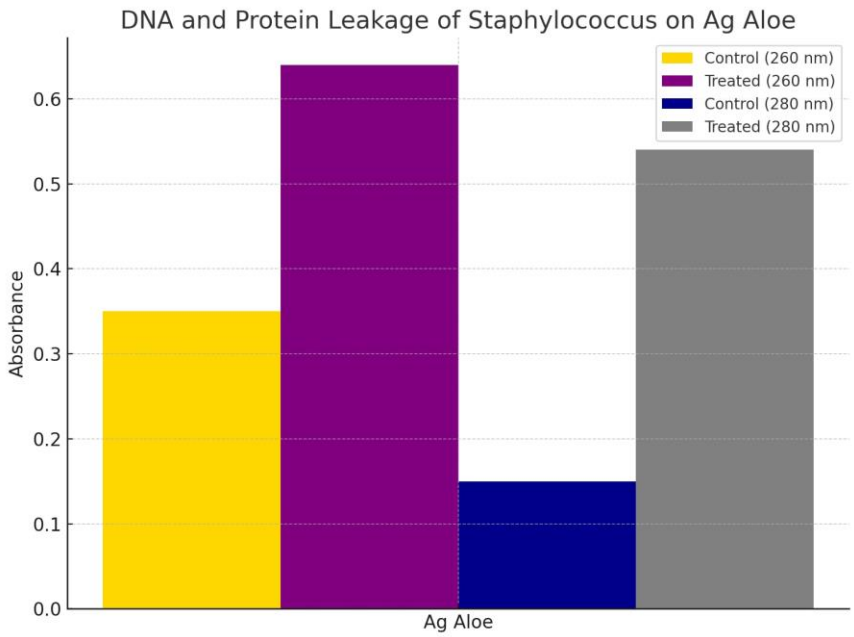


Fig 4.8. DNA and protein leakage of Staphylococcus treated with Ag Aloe

The bar graph illustrates the effects of Ag Aloe treatment on Staphylococcus DNA and protein leakage by measuring absorbance at 260 nm for DNA and 280 nm for protein.

After treatment, there was a noticeable increase in DNA leakage, as shown by the absorbance at 260 nm rising from about 0.35 (Control) to 0.64 (Treated).

At 280 nm, the absorbance rose significantly from 0.15 (Control) to 0.54 (Treated), suggesting a marked increase in protein leakage as well.

All things thought of, the results suggest that applying Ag Aloe reduces the bacterial barrier, allowing more intracellular material, including proteins and DNA, to escape.

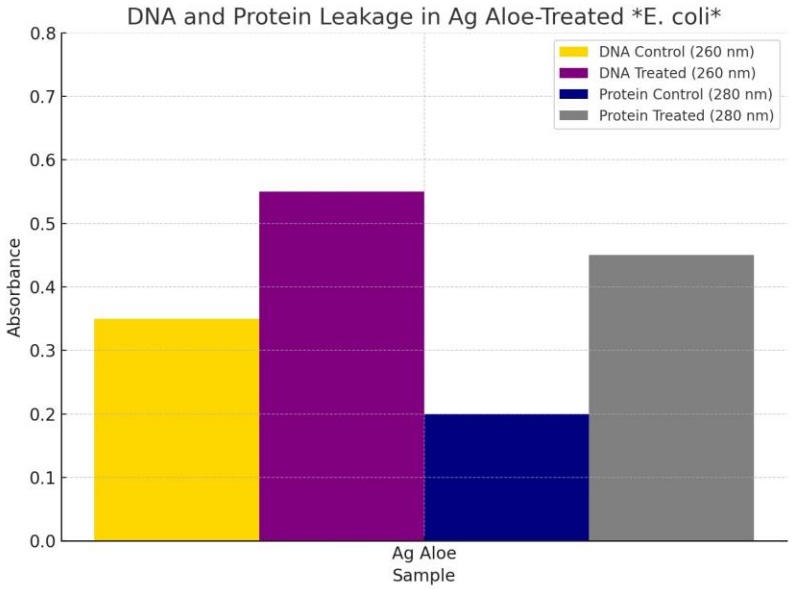


Fig 4.9. DNA leakage and protein leakage for *E.coli* treated with silver nanoparticles synthesized using Aloe vera extract

The graph, which measures protein and nucleic acid leakage, illustrates how Ag Aloe treatment impacts the integrity of the E. coli membrane:

DNA Leakage (260 nm): The absorbance (0.55) of the treatment group was significantly higher than that of the control group (0.35), indicating that a significant amount of DNA was released into the extracellular medium upon membrane breakdown.

Protein leakage (280 nm) was substantially greater in the treated sample (0.45) than in the control (0.20), which indicated the presence of cell lysis or increased membrane permeability. These findings prove that AgNPs made from aloe vera extract have antibacterial qualities by compromising the integrity of bacterial membranes, allowing intracellular macromolecules such as proteins and nucleic acids to leak out.

Conclusion

This study effectively demonstrates the green synthesis of silver nanoparticles (AgNPs) using extract from Aloe barbadensis Miller (Aloe vera), providing a sustainable and biocompatible substitute for conventional chemical synthesis techniques. The phytochemicals found in aloe vera, in particular flavonoids, phenolic compounds, and polysaccharides, were effective stabilizing and reducing agents when the nanoparticles were being formed. The partially amorphous nature, nanoscale size distribution, and successful production of AgNPs has been established by characterization studies using FTIR, XRD, DLS, and UV-Vis spectroscopy.
The generated nanoparticles suggested potent antibacterial activity against *Escherichia coli and Bacillus subtilis,* among other Gram-positive and Gram-negative bacteria. Increased protein and DNA leakage indicated obvious cellular membrane damage, and the outcomes of the agar well diffusion and broth microdilution tests demonstrated a dose-dependent inhibitory action.
These results confirm our recent finding that plant extracts, which are abundant in phytochemicals such flavonoids, terpenoids, and polyphenols, act as both stabilizing and reducing agents while the production of nanoparticles. The technology is an excellent alternative to traditional chemical synthesis methods due to its affordability, ease of use, and environmental friendliness.

Appendix

Sl. No.	Material/Equipment Used	Specification/Details
1	Aloe vera leaves	Fresh, botanically verified
2	Silver nitrate ($AgNO_3$)	0.01 mM aqueous solution
3	Distilled water	Double-distilled
4	Mortar and pestle	For mechanical extraction
5	Whatman No. 1 filter paper	For extract filtration
6	Magnetic stirrer	For uniform mixing
7	Conical flasks	For synthesis and incubation
8	UV-Vis Spectrophotometer	For surface plasmon resonance analysis
9	FTIR Spectrometer	For functional group analysis
10	X-ray Diffraction (XRD) Machine	For crystalline structure identification
11	Dynamic Light Scattering (DLS) Analyzer	For size distribution analysis
12	LB Broth/Nutrient Broth	For culturing bacteria
13	Agar media and Petri dishes	For agar well diffusion tests
14	Sterile cork borer	To create wells in agar plates
15	Incubator	37°C, for bacterial incubation
16	96-well microtiter plates	For broth microdilution assay

Table A1: Materials and instruments used in study

List of Bacterial Strains -

Sl. No.	Organism	Type	Gram Staining
1	*Escherichia coli*	Test organism	Gram-negative
2	*Bacillus subtilis*	Test organism	Gram-positive
3	*Staphylococcus aureus*	Test organism	Gram-positive

Spectral Peak -

Technique	Observed Peaks	Interpretation
UV-Vis	~425 nm	SPR indicative of AgNP formation
FTIR	3300, 1640, 1100 cm^{-1}	–OH, C=O, C–O groups (from Aloe vera)
XRD	Broad peak (20–35° 2θ)	Amorphous or nanocrystalline silver structures
DLS	~10–80 nm size distribution	Hydrodynamic diameter of AgNPs

Figure A3: Spectral peak observations and their interpretation

Visual Observation -

- **Color Change**: Pale yellow to dark brown – indicates successful AgNP formation.
- **Plate Images**: Clear zones around wells indicate antimicrobial activity.
- **UV-Vis Spectrum**: Peak at 425 nm confirms nanoparticle synthesis.
- **FTIR Shifts**: Reflect bonding of biomolecules with AgNP surfaces.

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